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14. ABSTRACT
Traumatic Brain Injury (TBI) is a well-established inducer of temporal lobe epilepsy (TLE), a frequently medically intractable and permanent epilepsy syndrome. Unlike many TLE models, which cause global brain injury that do not replicate the human condition, or other TBI models, which do not induce TLE reliably, the controlled cortical impact (CCI) model of posttraumatic epilepsy in mice results in localized cell loss, synaptic reorganization, and development of TLE. Abnormalities in inhibitory neurotransmission are important aspects of TLE in several animal models. Under this award, the CCI model was established in all three collaborating laboratories. Specific parameters of injury associated with epileptogenesis were determined. It was determined that upregulation of the JaK/STAT3 pathway in the injured hippocampus occurs after CCI, which could be blocked by post-injury administration of a JaK/STAT3 inhibitor. Blocking JaK/STAT3 activity did not prevent loss of GABA cells in the injured hippocampus. Inhibitory postsynaptic currents in the dentate gyrus ipsilateral to the injury were reduced in frequency weeks after the injury, recapitulating findings in other models in which aspects of epileptogenesis were attenuated by STAT3 inhibition. These results critically establish model parameters and control measurements, and provide the basis for remaining proposed experiments.

15. SUBJECT TERMS
dentate gyrus, epileptogenesis, GABA(A) receptor, JAK/STAT, posttraumatic epilepsy

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Progress Report Summary #W81XWH-11-1-0502 (July 2012-June 2013)

INTRODUCTION:

This research addresses the FY10 PRMRP topic area of Epilepsy. Traumatic Brain Injury (TBI) is a well-established etiology of temporal lobe epilepsy (TLE), a frequently medically intractable and often progressive epilepsy syndrome. Much evidence indicates that abnormalities in inhibitory neurotransmission are important in TLE. Our overall hypothesis is that Janus Kinase (JaK)/Signal Transducer and Activator of Transcription 3 (STAT3) pathway activation after TBI leads to GABA(A) receptor α1 subunit gene (*Gabra1*) repression and is a critical mediator of post-traumatic epileptogenesis and epilepsy progression. The JaK/STAT pathway has not been studied in post-traumatic epilepsy, but beyond its role in *Gabra1* regulation, it is known to be an important regulator of neuronal proliferation, survival and gliogenesis, all of which may be important contributors to epileptogenesis. Specifically, long-term decreases in expression of the GABA(A) Receptor α1 subunit gene (*Gabra1)* in the hippocampal dentate gyrus have been shown to occur in the pilocarpine-model of TLE in animals and preventing this repression has been shown to inhibit development of epilepsy in this model. In addition, decreases in expression of α4 subunits, which contribute to the generation of tonic GABA currents and potently regulate granule cell activity, have also been reported in TLE and after TBI. It has been recently established that transcriptional repression of GABA receptor subunits in the pilocarpine-model of TLE is mediated by inducible cAMP early repressor and phosphorylated CREB, and that ICER transcription is driven by the JaK/STAT signaling cascade. Pharmacological inhibition of the JaK/STAT3 pathway prevents *Gabra1* repression and inhibits progression of epilepsy in the pilocarpine model. Preliminary data indicated that spontaneous seizures activate the JaK/STAT3 pathway in the pilocarpine model, suggesting this pathway may be involved in the maintenance and progression of TLE. Preliminary evidence shows that the JaK/STAT3 pathway is activated following TBI in injured hippocampus and cortex after a *diffuse* injury, and data obtained under this award indicate that phosphorylated STAT3 is increased shortly after focal injury near the injury site, with concurrent changes in α1 and α4 GABA receptor subunit expression. The impact however, on GABA(A) receptor subunit function, and whether these are important mechanisms of post-traumatic epileptogenesis are unknown. Focal injury caused by controlled cortical impact (CCI) has been shown to induce cell loss, synaptic reorganization, and TLE in mice (Hunt et al., 2009; 2012). In order to assess changes in GABA responsiveness and alteration of those changes by Jak/STAT3 inhibition, it was necessary in the initial year of the study to coordinate procedures across three labs, establish that the fundamental changes in JaK/STAT3 activity occurred, and to ensure that the JaK/STAT3 blocker was effective in this model. We also examined the effect of STAT3 blockade on indicators of neuronal proliferation and survival. Specific experiments on critical aspects of GABA function were initiated in order to address the Specific Aims of the study. In the second year, as reported here, we continued examination of GABA receptor changes in the CCI-injured mouse and the effects of blocking STAT3 phosphorylation on those changes. Results of these studies will provide new information regarding the role of the JaK/STAT signaling cascade in regulation of brain inhibition and epileptogenesis after traumatic brain injury, and have the promise of leading to new therapies for the prevention or treatment of post-traumatic epilepsy.

BODY:

Aim 2: Performed in laboratory of Dr. Bret N. Smith at University of Kentucky
Determine whether activation of the JaK/STAT pathway and downregulation of *Gabra1* transcription following TBI result in altered inhibitory synaptic neurotransmission in the hippocampus that may contribute to epileptogenesis.

Task 1: Determine whether benzodiazepine modulation of IPSCs in dentate granule cells (DGCs) is altered after CCI and whether this alteration is prevented by inhibiting STAT3 phosphorylation with WP1066. (Timeframe months 1-18).

Task 1a. Induce TBI using CCI model in adult CD-1 mice (200 mice used, 20 sham-injured controls, 80 injured untreated, 20 sham-injured, WP1066-treated controls, 80 injured WP1066-treated; Timeframe months 1-18.
Status: In progress

1. Verified parameters of CCI injury that result in epileptogenesis and markers of the development of epileptic phenotype after 8-12 weeks post-injury. Approximately 30 mice were treated with CCI in the last year, 10 of which were injected with WP-1066. Based on collaborators findings that more severe injury results in greater STAT3 phosphorylation, studies included a few mice with greater injury.

a. The spatial extent of moderate and severe injury-related epileptogenic changes with respect to distance from impact point was initiated.

2. Establish that phosphorylation of STAT3 is upregulated and was inhibited by WP1066 in this model.

a. Establish effect and localization of STAT3 phosphorylation after CCI.
b. Establish effect of WP1066 on STAT3 phosphorylation after CCI.
c. Based on collaborators preliminary results, initiated studies to examine the extent of STAT3 phosphorylation after more severe injury. The results of this comparison will inform future electrophysiological analysis.

Accomplishments:

1. Determined precise parameters of effective CCI (1 mm depth) to obtain epileptogenic phenotype. Phenotype changes are currently being assessed for more severe injury (2 mm), based on preliminary findings from collaborators.
2. Completed determination that CCI increased STAT3 phosphorylation ipsilateral to the injury after 24 hours, but not contralaterally. Western blot analyses of hippocampi from CCI-injured and control mice were concluded after 1 mm depth injury. Both STAT and phosphorylated STAT (pSTAT) protein expression were compared semi-quantitatively 24 hr after injury (see progress report from year 1). Comparisons were made for hippocampi ipsilateral to the injury, contralateral to the injury, and in sham-operated controls. STAT and pSTAT levels were normalized to those for β-actin. Results indicated that pSTAT ($p<0.05$), but not STAT ($p>0.05$), expression was increased in the hippocampus ipsilateral to the injury. We further determined that treatment at 30 and 90 min after CCI (1 mm) with WP1066 (50 mg/kg) inhibits STAT3 phosphorylation in mice. Full analysis was reported (Butler et al., 2012; Boychuk et al., 2012). Conclusion: The biochemical reaction required to perform further analyses of GABA currents is evident after CCI ipsilateral to the 1 mm injury, but not contralaterally. This means that the contralateral dentate gyrus can serve as a control for electrophysiological analyses. Determining that pSTAT3 expression for 2 mm depth is underway.
3. Completed analysis of histopathological features (i.e., MFS and hilar GABA neuron loss) in the dentate gyrus consistent with epileptogenesis. Mossy fiber sprouting analysis was completed in year one (Hunt et al., 2012). Assessment of hilar inhibitory neuron loss was

completed in year 2, but preliminary data was included in the previous progress report (Butler et al., 2012; Boychuk et al., 2012). The distribution of inhibitory neuron loss was compared to previous results on mossy fiber sprouting. Significant GABA neuron loss was limited to the injury epicenter and an area extending 800 μm temporal (ventral) to the injury; areas septal (dorsal) to the injury were unaffected, similar to the distribution of mossy fiber sprouting. Contralaterally and at more ventral levels ipsilaterally, hippocampal pathology was not observed (Butler et al., 2012). Ongoing studies include more severe (2 mm) injury depth. We further determined that treatment with WP1066 does not affect GABA neuron loss. Full analysis of mice from the two injection paradigm (50 mg/kg each), delivered at 30 and 90 min post-injury (1 mm) was completed. The WP1066 treatment did not, however, alter the degree of GABAergic hilar interneuron cell loss ipsilateral to the injury (Butler et al., 2012). Conclusions: The histopathology most likely to be required for studying changes in GABA currents after CCI was associated with hippocampal evulsion, which was observed when using beveled tips (versus rounded tips) to a depth of 1 mm. Treatment with WP1066 showed no evidence of influence on cell proliferation, axon growth, or neuronal survival, Effects of 2 mm depth are in progress.

Supporting Data: Figures 1 & 2 from final progress report, year 1 summarize: 1. Western blot results indicating increased STAT3 phosphorylation after 1 mm CCI ipsilateral to the injury and inhibition of pSTAT3 by systemic WP1066 treatment (50 mg/kg, i.p.) at 30 and 90 min after CCI injury; 2. the hilar GABA neuron loss after CCI and the lack of effect of WP1066 treatment on hilar neuron loss. These data were completed in year 2, but reported in revised progress report for year 1.

Task 1b. Measure effects of zolpidem on IPSCs in DGCs from WP1066-treated and untreated control mice and in mice shortly (i.e., 1-6 weeks) after CCI injury. (100 mice needed, 10 sham-injured controls, 40 injured untreated, 10 sham-injured, WP1066-treated controls, 40 injured WP1066-treated; subset of mice in Task 1a; Timeframe months 1-9).

Status: In progress

Accomplishments:

1. IPSCs were recorded from granule cells from sham-(n=2) and CCI-treated (n=3) mice (1-6 weeks post-injury). IPSC frequency, amplitude, and decay time constants were measured. Analysis is preliminary and ongoing.

2. Applied α1-subunit selective benzodiazepine agonist and measured IPSC frequency, amplitude and decay time constant in neurons from control (n=2) and CCI-treated (n=3) mice. Analysis is preliminary: benzodiazepine agonist tended to increase time constant; replicates are currently too low to be quantitative.

Supporting Data: None

Task 1c. Measure effects of zolpidem on IPSCs in DGCs from WP1066-treated and untreated control mice and in mice 6-10 weeks after CCI injury. (100 mice needed, 10 sham-injured controls, 40 injured untreated, 10 sham-injured, WP1066-treated controls, 40 injured WP1066-treated; subset of mice in Task 1a; Timeframe months 4-18)

Status: In progress

1. Establishing precise IPSC parameters in control and CCI-injured mice at 6-10 weeks after injury. This includes:

a. Train personnel in recording and analysis techniques.
b. Obtain sufficient numbers of recordings to sufficiently identify differences

2. Effects of zolpidem on IPSCs in four treatment groups.

a. Obtain DEA license to purchase benzodiazepine agonists

b. Use zolpidem or other α1 subunit agonist in recordings from DGCs in slices from four treatment groups; 6-10 weeks postinjury.

Accomplishments:
1a. All personnel trained
1b. Preliminary data suggest a trend toward longer IPSC decay time constants in CCI-injured mice, 6-10 weeks after injury. These experiments continue and should be completed by August 2013. Experiments on CCI-injured+WP1066-treated mice are scheduled.
1. Effects of α1 subunit-selective agonist on IPSC frequency, amplitude, and decay time constant have been initiated in cells from sham control and CCI-injured mice.

Supporting Data: See **Table 1** below for preliminary assessment of IPSC amplitude, frequency, and time constant for control, CCI-injured, and CCI-injured+WP1066 treated mice.

Task 1d. Perform Timm histological analysis, to detect mossy fiber sprouting in all slices from which recordings are made. (200 mice needed; same mice as in Tasks 1a-c; Timeframe months 1-18).

Status: In progress

Accomplishments:
1. in year 2, approximately 20 mice were treated with CCI, 10 of which were treated with WP1066.
2. Early post-injury experiments are underway; Timm staining has not revealed mossy fiber sprouting in any group at this time-point, as expected.
3. At 6-10 weeks post-injury, Timm staining was observed in CCI-injured, but not sham control mice. CCI-injured+WP1066 treated mice displayed Timm distribution similar to CCI-injured mice at 6-10 weeks post-injury.

Supporting Data: Timm scores for the three groups analyzed: Control= 0.13 x ± 0.09 (n=16); CCI-injured= 1.93 ± 0.30 (n=16; $p<0.05$ vs control); CCI-injured+WP1066= 1.88 ± 0.30; (n=16; $p<0.05$ versus control; $p>0.05$ versus CCI-injured; ANOVA). Mossy fiber sprouting was not reduced by WP1066 treatment after CCI injury. See **Figure 1** below.

Task 2: Determine if furosemide modulation of IPSCs in DGCs is altered after CCI and if inhibiting STAT3 phosphorylation with WP1066 prevents the alteration. (Timeframe: months 19-36)

Task 2a. Induce TBI using CCI model in adult CD-1 mice (200 mice used, 20 sham-injured controls, 80 injured untreated, 20 sham-injured, WP1066-treated controls, 80 injured WP1066-treated; Timeframe months 19-36).

Status: in progress
Accomplishments: Accomplishments identical to Task 1, 1a.
Supporting Data: Same as Task 1, 1a.

Task 2b. Measure effects of furosemide on IPSCs in DGCs from WP1066-treated and untreated control mice and in mice 1-6 weeks after CCI injury. (100 mice needed, 10 sham-injured controls, 40 injured untreated, 10 sham-injured, WP1066-treated controls, 40 injured WP1066-treated; subset of mice in Task 2a; Timeframe months 19-27).

Status: In Progress.
Accomplishments: Experiments have been made in DGCs from in 5 mice.
Supporting Data: none

Task 2c. Measure effects of furosemide on IPSCs in DGCs from WP1066-treated and untreated control mice and in mice 6-10 weeks after CCI injury (months 4-18). 100 mice needed, 10 sham-

injured controls, 40 injured untreated, 10 sham-injured, WP1066-treated controls, 40 injured WP1066-treated; subset of mice in Task 2a; Timeframe months 19-36).

Status: In progress

Accomplishments: Experiments have been initiated. IPSC parameters have been established as in Task 1c.

Supporting Data: Table 1.

Task 2d. Perform Timm histological analysis, to detect mossy fiber sprouting in all slices from which recordings are made. (200 mice needed; same mice as in Tasks 2a-c; Timeframe months 19-36).

Status: in progress

Accomplishments: Accomplishments identical to Task 1, 1d.

Supporting Data: Same as Task 1, 1d.

Task 3: Determine if THIP-induced tonic GABA currents in DGCs are altered after CCI and if the alteration is prevented by inhibiting STAT3 phosphorylation with WP1066. (Timeframe months 10-27)

Task 3a. Induce TBI using CCI model in adult CD-1 mice (200 mice used, 20 sham-injured controls, 80 injured untreated, 20 sham-injured, WP1066-treated controls, 80 injured WP1066-treated; Timeframe months 10-27)

Status: in progress

Accomplishments: Accomplishments identical to Task 1, 1a.

Supporting Data: Same as Task 1, 1a.

Task 3b. Measure THIP-induced tonic GABA current in DGCs from WP1066-treated and untreated control mice and in mice 1-6 weeks after CCI injury. (100 mice needed, 10 sham-injured controls, 40 injured untreated, 10 sham-injured, WP1066-treated controls, 40 injured WP1066-treated; subset of mice in Task 3a; Timeframe months 10-18).

Status: In progress

Accomplishments: 4,5,6,7-Tetrahydroisoxazolo[5,4*c*]pyridine-3-ol hydrochloride (THIP) currents have been measured in 7 cells from 7 controls and 6 cells in 5 CCI-treated mice.

Supporting Data: Same as Task 1, 1a.

Task 3c. Measure THIP-induced tonic GABA current in DGCs from WP1066-treated and untreated control mice and in mice 6-10 weeks after CCI injury (months 4-18). 100 mice needed, 10 sham-injured controls, 40 injured untreated, 10 sham-injured, WP1066-treated controls, 40 injured WP1066-treated; subset of mice in Task 3a; Timeframe months 19-27).

Status: in progress

Accomplishments:

1. Determined that THIP-induced tonic GABA current in DGCs 6-10 weeks post-injury are reduced in amplitude relative to sham-operated controls and contralateral DGCs (n=7-14 cells in 7 mice from each group; $p<0.05$). Based on preliminary results from collaborators in Colorado and on data published recently elsewhere, these experiments were initiated to identify potential functional changes due to altered α1 vs α 4/δ subunit-containing GABA receptor expression weeks after injury, corresponding to time points where epilepsy is established in this model (i.e., 6-10 weeks post-injury). Granule cells were recorded in ex vivo slices taken from control mice and from slices taken contralateral and ipsilateral to injury site in CCI-injured mice, 6-10 weeks post-injury. Cells were voltage-clamped at 0 mV and THIP (3μM) was bath-applied to induce an outward current, ostensibly due to activation of δ subunit-containing GABA receptors (most likely α4/δ). Bicuculline methiodide (30 μ M) was applied to block all GABA receptors and determine the total available tonic GABA current. Conclusions: Total tonic GABA current in granule cells from control versus CCI-injured mice. THIP-current in cells contralateral to the injury are not different from those in control mice. However, THIP-induced tonic current is reduced

by about 50% ipsilateral to the injury in cells from CCI-treated versus controls ($p<0.05$) or in cells contralateral ($p<0.05$) to the injury.

2. Recordings were made from 12 granule cells in 7 WP1066-treated CCI-injured animals and tonic GABA and THIP currents were compared to results from control mice and CCI-injured mice. As in other groups, tonic GABA current was unaffected in WP1066-treated CCI-injured mice ($p>0.05$), as expected. The THIP-current was significantly reduced by about 50% ipsilateral to the injury in cells from WP1066+CCI-treated versus controls or in cells contralateral to the injury ($p<0.05$). The THIP current in the WP1066+CCI-injured group was not different from CCI-treated group ($p>0.05$).
3. Control studies are ongoing to determine if WP1066 treatment affects non-injured controls.

Supporting Data: see **Figure 2 below** for demonstration of THIP-induced changes in tonic GABA current in sham-operated controls, CCI-injured, and CCI-injured+WP1066 after 6-10 weeks post injury.

Task 3d. Perform Timm histological analysis, to detect mossy fiber sprouting in all slices from which recordings are made. (200 mice needed; same mice as in Tasks 3a-c; Timeframe months 10-27).

Status: in progress

Accomplishments: Accomplishments identical to Task 1, 1d.

Supporting Data: Same as Task 1, 1d.

KEY RESEARCH ACCOMPLISHMENTS:

- Continued to refine precise injury parameters for CCI in mice that yield consistent and reliable outcome measures. All tasks require the CCI model to be established. Began comparing 1 mm (moderate) versus 2 mm (severe) injury.
- Determined that 1 mm injury causes sufficient damage to induce pSTAT3 production to initiate hypothesized signal cascade necessary to result in epileptogenic phenotype.
- Completed training of all lab personnel on project.
- Phosphorylated STAT3 levels were increased in the hippocampus ipsilateral to the injury 24 hours after CCI. By one week, there was no appreciable continued activation.
- In mice, 30 and 90 minute post-treatment of WP1066 inhibits phosphorylation of STAT3 in injured hippocampus 24 hours after CCI to control levels. All tasks require controls to demonstrate effectiveness of WP1066.
- In mouse, mossy fiber sprouting in the inner molecular layer is regionally and locally enhanced after CCI in a semi-quantitatively measurable manner. STAT3 inhibition did not affect mossy fiber sprouting 6-10 weeks post-injury.
- Selective hilar GABA interneuron loss was documented near the injury site after CCI-injury. STAT3 inhibition did not alter the cell loss.
- Total tonic GABA currents were not significantly altered in granule cells of the dentate gyrus ipsilateral to the injury 6-10 weeks after CCI in mice. However, THIP-induced tonic currents were reduced ipsilateral to the injury by 6-10 weeks after CCI-injury. Inhibition of STAT3 with WP1066 at the time of CCI-injury did not reinstate the THIP-activated tonic current ipsilateral to the injury.

REPORTABLE OUTCOMES (not previously reported): manuscripts, abstracts, presentations

1. D Raible, L Frey, J Boychuk, C Butler, H Grabenstatter Y Cruz Del Angel, S Russek, B Smith and A Brooks-Kayal. JaK/STAT inhibition to prevent posttraumatic epilepsy. Poster Presentation. Rocky Mountain Regional Neuroscience Group Annual Meeting, UC AMC. 2013

2. D Raible, L Frey, J Boychuk, C Butler, H Grabenstatter Y Cruz Del Angel, S Russek, B Smith and A Brooks-Kayal. JaK/STAT inhibition to prevent posttraumatic epilepsy. Poster Presentation. CTSA national pre-doctoral meeting. 2013

3. D Raible, L Frey, J Boychuk, C Butler, H Grabenstatter Y Cruz Del Angel, S Russek, B Smith and A Brooks-Kayal. JaK/STAT inhibition to prevent posttraumatic epilepsy. Poster Presentation. Department of Neurology Research Retreat. 2013

4. D Raible, L Frey, J Boychuk, C Butler, H Grabenstatter Y Cruz Del Angel, S Russek, B Smith and A Brooks-Kayal. JaK/STAT inhibition to prevent posttraumatic epilepsy. Oral Presentation. Rocky Mountain Regional Neuroscience Group Annual Meeting. 2013

5. Butler, CB, Boychuk, JA, Smith, BN. Inhibitory signaling to dentate granule cells following traumatic brain injury. *Soc. Neurosci. Abs.*, 39:in press. This abstract describes results of tonic GABA modulation by THIP in control and CCI-injured mice.

CONCLUSIONS

In the second year of DOD CDMRP funding, the CCI model was refined in mice at both University of Colorado and University of Kentucky, making future experiments feasible. Essential baseline control data was obtained to ensure that all aspects of CCI-injury and of WP1066 treatment were feasible and repeatable. Notably, 1 mm injury depth resulted in STAT phosphorylation, inhibitory cell loss, mossy fiber sprouting, and reduction in THIP-activated tonic GABA currents. A significant percentage of these animals were confirmed to express behavioral seizures. Colleagues at the University of Colorado are having success with a 2 mm injury, so effects of this more severe injury on the parameters we study are underway to determine if an injury severity-response relationship exists. CCI-injury significantly phosphorylates STAT3 after injury and treatment with WP1066 significantly reduced this phosphorylation. Select GABA neuron loss is seen shortly after injury, and mossy fiber sprouting develops after several weeks post-injury, neither of which outcome was altered in WP1066-treated mice. Total tonic GABA currents in granule cells are unaffected by the injury. However, contrary to some other models, THIP-activated tonic GABA currents are reduced in granule cells ipsilateral to the injury, suggesting a reduction in δ GABA receptor subunits (possibly α4/δ-containing), as hypothesized. Treatment with WP1066 did not reinstate the reduction in THIP-activated current, suggesting that the decrease in δ-subunit containing GABA receptors after injury is not affected by STAT3 inhibition. Previously, Raible et al (2012) showed that α4-subunits are decreased one week after fluid percussion injury. Often, α4-subunits pair with δ-subunits. The reduction in THIP-activated current is consistent with this finding. It is also consistent with a lack of influence of STAT3 phosphorylation on δ-subunit expression. Specific α4-subunit associated effects will be the target of experiments in aim 2 in the coming year. Alterations in α1 subunit function will be the priority focus of experiments over the coming months. No specific changes in the proposed experiments are suggested.

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APPENDICES

SUPPORTING DATA:

Table 1. IPSC frequency, amplitude and decay time constant in control, CCI-injured and CCI-injured+WP1066 treated mice.

sIPSCs	Sham		CCI				CCI+WP1066			
			Contralateral		Ipsilateral		Contralateral		Ipsilateral	
	Baseline	THIP	Baseline	THIP	Baseline	THIP	Baseline	THIP	Baseline	THIP
Frequency(Hz)	1.41±0.09	0.79±0.16	1.30±0.26	0.82±0.08	0.80±0.12	0.68±0.08	1.33±0.34	0.74±0.35	0.72±0.09	0.69±0.07
Peak Amplitude(pA)	20.36±1.92	21.02±1.20	20.89±1.88	22.11±3.05	22.85±3.02	20.93±2.24	21.72±2.58	23.34±3.36	18.71±1.57	21.22±1.78
10-90% Rise Time (ms)	2.23±0.10	2.29±0.09	2.29±0.18	2.24±0.44	2.47±0.11	2.53±0.18	2.30±0.13	2.33±0.09	2.45±0.18	2.42±0.12
Decay Time Constant (ms)	14.06±3.40	13.28±1.86	15.33±3.26	14.17±2.02	17.04±3.56	16.03±1.63	14.10±0.67	13.35±0.88	16.97±1.49	15.70±2.05

Synaptic responses were assessed at baseline and at steady-state during 4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridine-3-ol hydrochloride (THIP; 3 µM) application. Sample sizes are sham injury (n=7), CCI injury (Contralateral: n=7, Ipsilateral: n=7) or CCI injury with acute WP1066 treatment (Contralateral: n=5, Ipsilateral: n=7). Data shown as mean ± SEM.

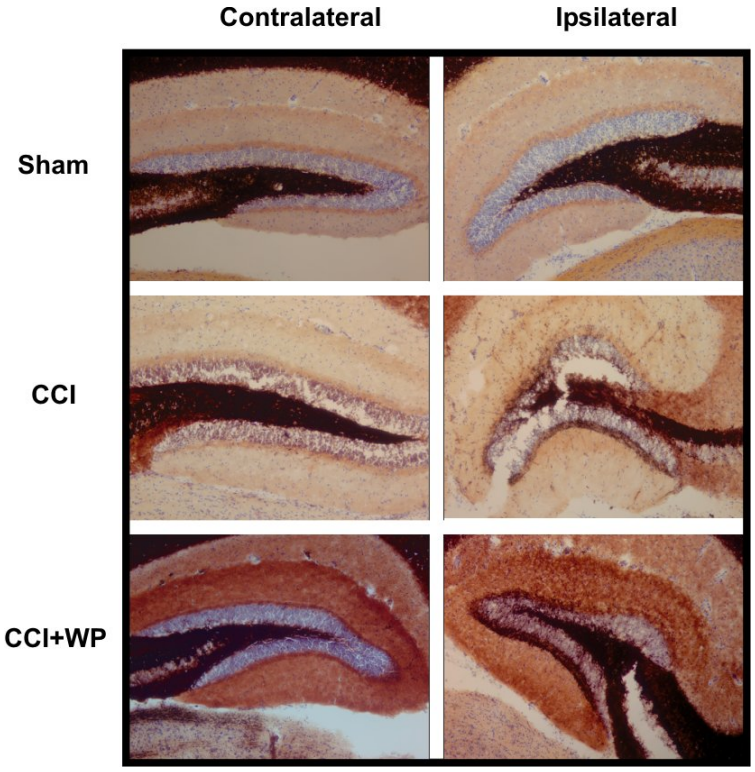

Figure 1. Timm staining in the dentate gyrus from sham control, CCI-injured, and CCI-injured+WP1066 treated mice. Images of the dentate gyrus contralateral (left images) and ipsilateral (right), directly under the 'epicenter' of the injury or skull opening in sham-operated controls, CCI-injured, or CCI-injured+WP1066-treated mice after ~8 weeks post-injury. Contralateral images are from approximately equivalent hippocampal levels contralateral to the skull opening. Mossy fiber sprouting into the inner molecular layer is evident ipsilateral to the injury in CCI-injured and CCI-injured+WP1066-treated mice and is similar to that seen previously to be associated with synaptic reorganization.

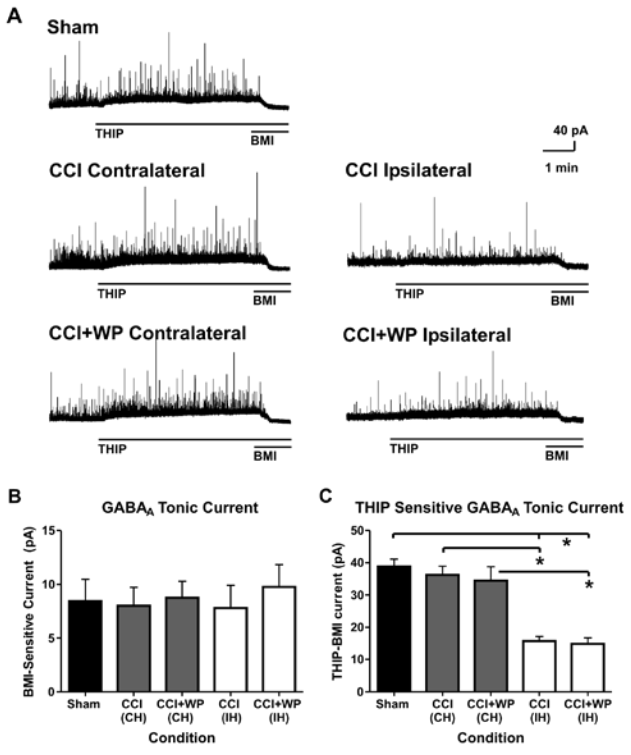

Figure 2. Reduced THIP-sensitive tonic $GABA_A$ currents in dentate granule cells (DGCs) located ipsilateral to controlled cortical impact (CCI) during 6-13 weeks post-injury. A. Representative traces of tonic $GABA_A$ currents in DGCs after sham injury (upper left; n=7), CCI injury (middle row; Contralateral: n=7, Ipsilateral: n=7) or CCI injury with acute WP1066 treatment (bottom row; Contralateral: n=5, Ipsilateral: n=7). DGCs were voltage clamped at 0 mV (near reversal potential of glutamatergic currents) and recorded in three phases: baseline, THIP (3 µM; Sigma, USA) and Bicuculline Methiodide (BMI; 30 µM; Tocris, USA). B. Group data of tonic $GABA_A$ currents measured as the change in steady-state holding current values of baseline to BMI application (Baseline I_{Hold} − BMI I_{Hold}). C. Group data of THIP-sensitive tonic $GABA_A$ currents measured as the change in steady-state holding current values of THIP application to bicuculline application (THIP I_{Hold} − BMI I_{Hold}). Given the recoding parameters here, an increase in tonic $GABA_A$ receptor-mediated currents using THIP produced an outward shift whereas blockade of $GABA_A$ receptors with BMI produced an inward shift in the holding current. Data shown as mean ± SEM and n= # of cells.

Appendix item: SFN Abstract

1) Butler, CB, Boychuk, JA, Smith, BN. Inhibitory signaling to dentate granule cells following traumatic brain injury. *Soc. Neurosci. Abs.*, 39:in press. This abstract describes results of tonic GABA modulation by THIP in sham-control and CCI-injured mice.

Inhibitory signaling to dentate granule cells following traumatic brain injury

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Traumatic brain injury (TBI) is one of the most common causes of temporal lobe epilepsy (TLE). Changes in inhibitory signaling after CCI include hilar inhibitory neuron loss, synaptic reorganization, and GABA receptor subunit reorganization, all of which may support the development of spontaneous seizures following TBI. Here, a mouse model of posttraumatic epilepsy using controlled cortical impact (CCI; velocity= 3.5m/s; duration= 500 msec; depth= 1.0mm) was used to examine inhibitory signaling within the dentate gyrus 8-13 weeks after injury. Tonic and phasic $GABA_A$ receptor-mediated responses were assessed using whole cell patch-clamp recordings of dentate granule cells (DGCs) in slices from control and injured mice. Both tonic and phasic $GABA_A$ responses were studied in voltage clamp at baseline and during steady-state bath application of 4,5,6,7-tetrahydroisoxa-zolo[5,40c]pyridin-3-ol (THIP) (3 μM) followed by bicuculline (30 μM) to explore δ subunit containing $GABA_A$ receptor function in DGCs after TBI.

Preliminary results indicate no significant difference in the total basal tonic current uncovered with bicuculline application in DGCs between CCI-injured and sham-operated control mice, or between ipsilateral and contralateral hippocampi of injured mice. However, the tonic current in DGCs from the injured hemisphere of CCI mice exhibited reduced sensitivity to THIP, suggesting a decreased contribution of δ subunit-containing $GABA_A$ receptors to tonic current generation. No significant differences between CCI-injured mice and sham-operated controls were detected in spontaneous inhibitory postsynaptic current (sIPSC) amplitude or kinetics, either at baseline or during THIP application. Results suggest that tonic $GABA_A$ receptor-mediated currents are reduced ipsilateral to the injury after CCI in mice, a response that involves reduced activity of δ subunit-containing $GABA_A$ receptors several weeks after injury. Ongoing studies will assess inhibitory signaling in DGCs at earlier time points after injury.

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